Bull. Inst. Zool., Academia Sinica **30**(1): 1-8 (1991)

ACTION OF NEUROACTIVE CHEMICALS ON SEX PHEROMONE-MEDIATED BEHAVIOR OF THE GERMAN COCKROACH, *BLATTELLA GERMANICA* (L.) (ORTHOPTERA: BLATTELLIDAE)

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(Accepted May 22, 1990)

Shuenn-Jue L. Wu and Judd O. Nelson (1991) Action of neuroactive chemicals on sex pheromone-mediated behavior of the German cockroach, *Blattella germanica* (L.) (Orthoptera: Blattellidae). *Bull. Inst. Zool. Academia Sinica* **30**(1): 1-8. The effects of several neuropharmacological agents and insecticides on sex pheromone-mediated behavior of male German cockroaches, *Blattella germanica* (L.), were investigated. A screening assay was adapted from the bioassay method developed for isolating and identifying the natural sex pheromone from female German cockroaches. Test conditions were optimized and standardized for the screening assay. Wing-raising behavior of male German cockroaches in response to pheromone stimulation was inhibited by pretreatment with either sublethal doses of insecticides or selected neuropharmacological agents. The insecticide chlordimeform inhibited the wing-raising behavior of males in a dose-dependent manner.

Key words: Behavior, German cockroach, Neurotoxicant, Sex pheromone.

Active components of the female sex pheromone of the German cockroach, *Blattella germanica* (L.), were identified as 3, 11-dimethyl-2-nonacosanone (compound A) and 29-hydroxy-3, 11-dimethyl-2-nonacosanone (compound B) (Nishida *et al.*, 1974, 1976; Jurenka *et al.*, 1989). The courtship behavior of males elicited by a sex pheromone-impregnated antenna consists of antennal fencing, 180° turning and wing-raising (Bell *et al.*, 1978b). The wing-raising behavior of males was used as an indicator of synthetic female sex pheromone activity in bioassays (Nishida *et al.*, 1974, 1976).

Our research objective was to develop a screening procedure for neuropharmacological agents and insecticides which might interfere with the sex pheromone-mediated behavior of the male German cockroach. Bell *et al.* (1978a) described factors affecting the response of male German cockroaches to the synthetic sex pheromone which included isolation of males, photoperiod and sensory habituation. Our initial effort was to optimize and standardize test conditions for the male response to the synthetic sex pheromone. The activity of chemicals were then screened using these standardized and optimized conditions.

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MATERIALS AND METHODS

Cockroach Maintenance

Cockroaches were maintained under a 12 hr light: 12 hr dark photocycle at 27°C and 50% relative humidity with a constant supply of food (Purina Lab Chow Pellets) and water. Newly molted adult males were isolated from females in a separate incubator. Male cockroaches were tested in groups of 5 in 18.5×18.5 cm plastic jars. The sides of the jars were coated with a 1:1 mixture of mineral oil and petroleum jelly to prevent escape. All tests were performed in male cockroach-conditioned containers (*i. e.* fecal spots produced by males were present). Red light bulbs (25 watt, GE) were used to illuminate the observation incubator.

Methods for Presentation of Stimuli by Pheromones

Compounds A and B of the *Blattella germanica* female sex pheromone were supplied by Prof. Albert W. Burgstahler, Department of Chemistry, University of Kansas, U. S. A. Stock solutions of these two compounds were prepared in CCl_4 at 10 mg/ml and serial dilutions of 1:10, 1:100, 1:1000 were prepared and stored at 4°C.

Methods employed for bioassays were modified from Bell *et al.* (1978a, b). Antennae used were ablated from either the adult male American cockroaches (*Periplaneta americana*) or the Madeira cockroaches (*Leucophaea maderae*). Freshly ablated antennae were attached to 20 cm disposable pasteur pipettes with white glue. These antennae were dipped in pheromone solutions for 1-2 sec and the solvent was then allowed to evaporate for 30 sec before use. The treated antennae were used to touch antennae of the 5 males in a test jar. Positive responses were recorded by the observation of wing-raising in individuals within 5 min. A fresh antenna preparation was used for each separate 5 min test period.

Optimizing Bioassay Conditions

Preliminary experiments were conducted to define factors such as the effects of male isolation, photoperiod, compound A versus B, pheromone concentration, selection of antennae to present the pheromone, and the effect of CO_2 anesthesia on the male pheromone response.

The effect of isolation on the response of males was determined using males which had been isolated from females for 2, 4, 6 to 20 days prior to the assay.

Bioassays were performed at hourly intervals after the onset of scotophase to determine the temporal variation in response of male cockroaches to synthetic sex pheromone during scotophase.

The minimum pheromone concentration necessary to elicit wing-raising behavior in untreated adult males was determined by varying the concentrations of the two synthetic pheromones presented by the two different types of antennae (*i. e.*, from American or Madeira cockroaches).

The time required for recovery of response after CO_2 anesthesia following acetone treatment of the cockroach antennae was determined by testing the male response at different times after anesthesia and sham treatments. These experiments were performed under the optimum bioassay conditions determined above.

Preparation and Application of Chemicals

A series of neuropharmacological agents including cholinergic, anticholinergic, adrenergic, antiadrenergic, miscellaneous drugs and some insecticides were obtained from several chemical companies. These included representative drugs for each class. Serial dilutions of various drugs were prepared in either

distilled water or organic solvents depending on their solubilities. For screening of water soluble drugs, cockroaches were injected between abdominal sternites with 1 μl of drug solution while the controls were injected with 1 μl of distilled water. Organo-soluble drugs were applied topically to the abdominal sternites in 1 μl volumes of drug solution, or with the solvent only for the controls. A calibrated microapplicator (Instrumentation Specialities Company, Inc., Lincoln, NE) was used for all treatments.

Bioassays of Chemical Induced Changes in Behavior

Screening assays were performed 24 hrs after the drug treatment with CO_2 anesthesia. Three concentrations of each drug were tested and three groups consisting of 5 males each were replicated for each concentration under optimized conditions. The test conditions were as follows: (1) male cockroaches were isolated for 14 days; (2) tests were performed between 2 and 4 hrs after the onset of scotophase; (3) compound B was applied to the ablated American cockroach antennae at a concentration of 1 μg/ml as presentation of stimuli.

The ED_{50} of chlordimeform as an inhibitor of wing-raising behavior was determined using 5 groups of 5 males each for four chlodimeform concentrations under the optimized screening conditions.

RESULTS

Standardization of the Bioassay

The percentage of males exhibiting wing-raising (% WR) increased as the number of days of male isolation increased. The period of isolation needed to consistently evoke>90% WR was 14 days (data not shown).

There were two peaks of male response to synthetic sex pheromone during scotophase of the photocycle (Fig. 1). The first peak of WR response

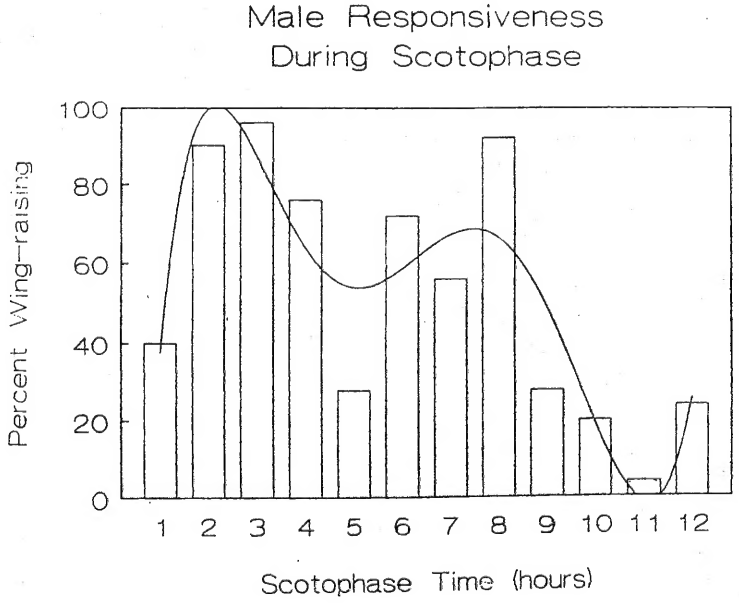

Fig. 1. Optimum responsiveness of male German cockroaches to synthetic sex pheromone during scotophase. Wing-raising is the behavioral response of males to female sex pheromone and is elicited by antennal "fencing" using pheromone-treated, isolated antennae. See text for assay conditions. The curve is a sixth order polynomial graph with a R value of 0.8921.

occurred between the second and third hours of scotophase. A second peak of WR response was observed 8 hours after the onset of scotophase. The first peak of male response was chosen as the optimum time to perform the screening assays.

Male cockroaches responded in a dose-dependent manner to two synthetic pheromones presented by two types of antennae (Fig. 2). Compound B was significantly more active than compound A (by about a hundredfold). Also, the ED_{50} of pheromones was significantly lower with the American cockroach antennae compared to the Madeira cockroach antennae.

The male wing-raising response at different times after solvent treatment with or without CO_2 anesthesia was investigated to optimize test conditions. The CO_2 anesthesia was found to delay the response of males to synthetic sex pheromone (data not shown), however, they fully recovered within 18 hours post treatment.

Effect of Chemicals on Wing-Raising Behavior

A rating system for the screening assay was based on both the drug dose and the behavioral response of male cockroaches as expressed in a percentage of males exhibiting wing-raising behavior in response to pheromone stimulation (see footnote in Table 1.).

The results show that neuropharmacological agents from several classes were active in interfering with sex pheromone-mediated behavior (Table 1). A rating of active classes of compounds that inhibited the wing-raising behavior was as follows: aminergic>antiadrenergic> GABA drugs > cholinergic > adrenergic> anticholinergic drugs.

The formamidine insecticide, chlordimeform, was the most effective chemical tested. A dose-response relationship was

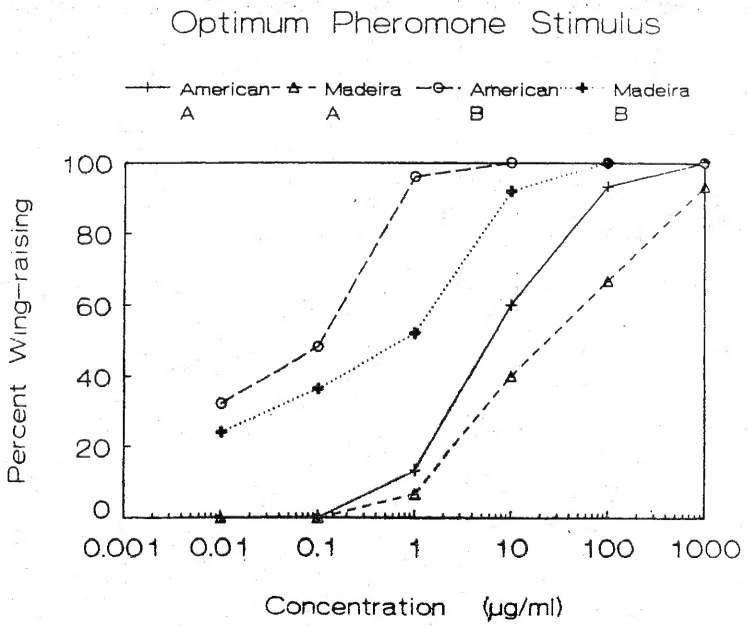

Fig. 2. Male wing-raising responses to two synthetic pheromones presented on two different types of isolated antennae. Compound A: 3, 11-dimethyl-2-nonacosanone. Compound B: 29-hydroxy-3, 11-dimethyl-2-nonacosanone. Assays were performed using 3 groups of 5 males each for each concentration. Tests were conducted 2 to 4 hours into scotophase using males which had been isolated for 14 days.

Table 1
Effects of different classes of neuroactive chemicals
on sex pheromone-mediated behavior
in male German cockroaches

Chemical	Rating	Chemical	Rating
Cholinergic		**Antiadrenergic**	
Acetylcholine chloride	+	Phentolamine HCl	##
Carbachol chloride	##	Guanethidine sulfate	##
Chlorpyriphos	+	Methyldopa	+
Anticholinergic		**Aminergic**	
Atropine	+	Octopamine HCl	+
Scopolamine HBr	+	Chlordimeform HCl	###
Adrenergic		5-Hydroxytryptophan	##
Isoproterenol HCl	+	**GABAergic**	
Tyramine	+	Gamma aminobutyric acid	+
Amphetamine sulfate	+	Muscimol	##
Ephedrine HCl	+	Bicuculine methiodide	+

Test conditions: Synthetic compound B solution (1 μg/ml) was presented to male German cockroaches on isolated American cockroach antennae at 2-4 hours into scotophase. Neuroactive chemicals were injected or applied topically as indicated at 24 hours before the behavioral assay. At least 2 groups of 5 males each were tested at chemical concentrations of 10^{-2}, 10^{-3} and 10^{-4} M. The following criteria were used: (1) no effect (−): 10^{-4} M gave >75% WR, 10^{-3} M gave >65% WR, or 10^{-2} M gave >55% WR, (2) slight effect (+): 10^{-4} M gave <75% WR, 10^{-3} M gave <65% WR, 10^{-2} M gave <55% WR, (3) moderate effect (##): 10^{-4} M gave <60% WR, 10^{-3} M gave <50% WR, 10^{-2} M gave <40% WR and (4) strong effect (###): 10^{-4} M gave <45% WR, 10^{-3} M gave <30% WR, 10^{-2} M gave <15% WR.

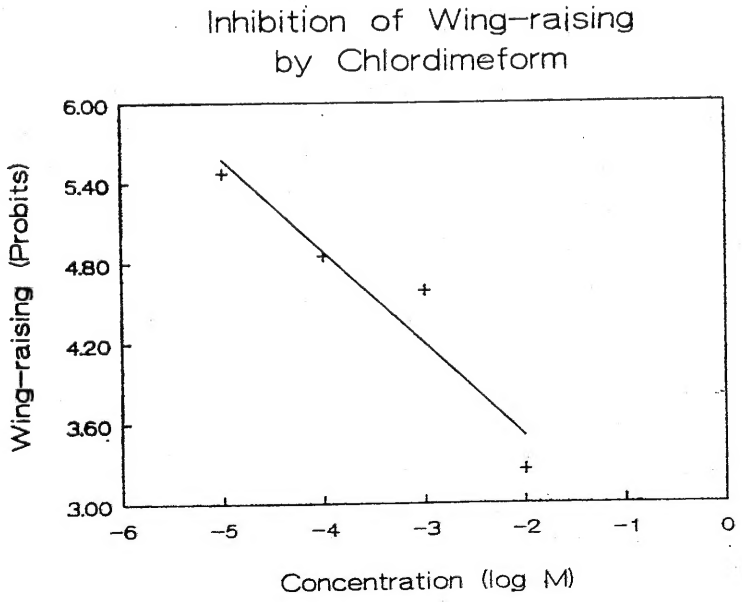

Fig. 3. The inhibition of male responsiveness to female sex pheromone by chlordimeform. Male cockroaches had been treated topically with chlordimeform 24 hours prior to the wing-raising behavioral assay.

demonstrated and ED_{50} of chlordimeform was determined to be 8.0×10^{-5} M which corresponds to a dosage of 0.37 μg/g (Fig. 3).

DISCUSSION

The response of male German cockroaches to synthetic sex pheromone is influenced by many physiological and environmental factors (Bell *et al.*, 1978a). Therefore, screening conditions such as, duration of male isolation, periods between treatment and testing, time of testing during the photocycle, components of pheromone, type of antennae used, and environmental conditions were standardized and optimized.

Containers having feces of male cockroaches modified the response of males to the sex pheromone. Bell *et al.* (1978a) also used feces-conditioned containers for bioassays to avoid the German cockroaches' indifference and restlessness previously observed by Takahashi and Kitamura (1972). It is likely that an aggregation pheromone plays a role in the response of males to the sex pheromone.

Bell *et al.* (1978a) reported that the response of males to the sex pheromone was significantly greater during scotophase than photophase. There is also a variation in responsiveness of males to the synthetic sex pheromone within scotophase (Fig. 1). Hawkins and Rust (1977) reported similar circadian variations in the activity of male American cockroaches to female sex pheromone.

The higher activity of compound B compared to compound A as shown in Figure 2 agrees with Nishida *et al.* (1976a, b) who found that compound B at a concentration of 3 μg/ml was about ten times more active than compound A on males isolated for 1-2 weeks. On the other hand, Burgstahler *et al.* (1977) reported that in assays on males isolated for 2-4 days, compound B at a concentration of 250 μg/ml elicited about half the activity of compound A. This discrepancy in thresholds to compounds A and B may be due to using males with different isolation periods.

Antennae of the American cockroaches are generally much thinner and longer than those of the Madeira cockroaches, and it was observed that the formers' curl less after dipping in the pheromone/CCl_4 solution. This may account for the observation that the ED_{50} of pheromones was significantly higher when using the Madeira cockroach antennae rather than the American cockroach antennae (Fig. 2). Compound B at a threshold concentration of 1 μg/ml using the American cockroach antennae provided optimal conditions for detecting active chemicals in the screening assays. Greater than 90% WR was consistently observed in control groups, while the inhibitory effects of neuroactive agents were detectable at this threshold stimulus condition when compared with control groups.

This initial effort to find prototypes or classes of compounds which were active in interfering with sex pheromone-mediated behavior of male German cockroaches was based upon the results of drugs on mammalian neurotransmitter systems (Goth, 1978). In insects, there is evidence that acetylcholine, dopamine, norepinephrine, serotonin, GABA, glutamate and octopamine are transmitter candidates (Pichon, 1974; Leake and Walker, 1980).

Chlordimeform is an effective formamidine insecticide and acaricide. It is not only toxic to insects and acarines but also induces abnormal behaviors at sublethal doses. These behavioral effects include antifeeding, colony dispersal, repellancy, hyperactivity, tick detachment and disruption of mating behavior (Beeman and Matsumura, 1978). Several

studies have demonstrated that chlordimeform interacts directly with octopaminergic receptor sites (Evans and Gee, 1980; Hollingworth and Murdock, 1980; Orchard *et al.*, 1982; Gole *et al.*, 1983). In our results, chlordimeform had a much greater inhibitory effect than that of octopamine. This was possibly due to greater tissue solubility (Table 1). There was a dose-response relationship for the blockade of male wing-raising activity by chlordimeform (Fig. 3). The ED_{50} determined (0.37 μg/g) was much smaller than the LD_{50} determined by Beeman and Matsumura (1974) (30 μg/roach=560 μg/g) for the German cockroach.

Linn and Roelofs (1984) reported that the precopulatory sequence of behaviors exhibited by male oriental fruit moths is very sensitive to sublethal concentrations of a range of neuroactive compounds. They found that the male response after treatment with chlordimeform was affected at all phases of the behavioral response. Linn and Roelofs (1986) studied the modulatory effects of octopamine and serotonin on male sensitivity and periodicity of response to sex pheromone in the cabbage looper moth. They also had a similar long term goal which is to define a neuroactive compound that can be used as a behaviorally active insect control agent.

In summary, several classes of neuropharmacological agents and insecticides were active in interfering with sex pheromone-mediated behavior in the German cockroach. It is therefore suggested that the expression of sexual behavior of the male German cockroach is under control of, or modified by, several neurotransmitter systems—some being excitatory and some being inhibitory. Perhaps new modes of action for chemicals which interfere with the normal male cockroach courtship behavior can be used to offset or forestall the continuing problem of insecticide resistance. Resistance has been a particular problem in the case of the German cockroach (Nelson and Wood, 1982).

Acknowledgements: We are grateful to Dr. Michael Ma for his critical comments and suggestions on the manuscript. This research was supported by the College of Agriculture and Life Science, University of Maryland at College Park and the Maryland Agricultural Experimental Station Scientific Article No. A-3688, Contribution No. 6664.

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神經性藥劑對德國蜚蠊性費洛蒙反應行爲的影響

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數種神經藥劑和殺蟲劑對德國蜚蠊 (*Blattella germanica*) 性費洛蒙反應的影響是本文研究探討的主題。篩選有效藥劑的方法是由原用於提純鑑定雌性德國蜚蠊天然合成性費洛蒙的生物分析方法改善而來。試驗所得的最適宜和標準化的篩選條件如下：(1)雄性蜚蠊先與雌性蜚蠊隔離十四天；(2)篩選試驗是於黑暗期後二至四小時進行；(3)德國蜚蠊雌性費洛蒙化學成份 B (29-hydroxy-3, 11-dimethyl-2-nonacosanone) (濃度 1 μg/ml) 佔於切除的美國蜚蠊觸角是刺激性雄性德國蜚蠊性費洛蒙反應行爲的方法。雄性蜚蠊對性費洛蒙刺激反應而產生的展翅行爲，會因爲低劑量殺蟲劑或數種神經藥劑的預先處理而受到抑制。在研究的藥劑中，克戴美風 (Chlordimeform) 抑制雄蟲展翅求偶行爲的作用，是一種與劑量成正比的關係。